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Pantoja Munoz, Leonardo and Purchase, Diane and Jones, Huw and Feldmann, Jörg and Garelick, Hemda (2014) The mechanisms of arsenic bioremediation from water by the green microalgae *Chlorella vulgaris*. In: THE BNASS / TraceSpec Tandem Conference, 31 Aug - 04 Sep 2014, Aberdeen, Scotland, UK.

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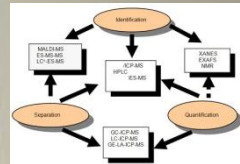
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# The Mechanisms of Arsenic bioremediation from water by the Green Microalgae *Chlorella vulgaris*



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In collaboration  
with



**TESLA**  
Trace element  
Speciation  
Laboratory  
Aberdeen

Leonardo Pantoja, Diane Purchase, Huw Jones,  
Jörg Feldmann and Hemda Garelick

# OUTLINE



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- ▶ Toxicity of arsenic to *C. vulgaris*
  - ASTM E1218 and flow cytometry
- ▶ Focused sonication for extraction for As-phytochelatin complexes
- ▶ Formation of As-GS/PC complexes as detoxification mechanism
  - Exposure to As(III) (Sodium arsenite)
  - Exposure to DMA(V) (Dimethylarsinic acid)
  - Exposure to As(V) (Sodium arsenate)
- ▶ Total As-GS/PC formation
- ▶ Transport of As-GS/PC to vacuoles
- ▶ Conclusions

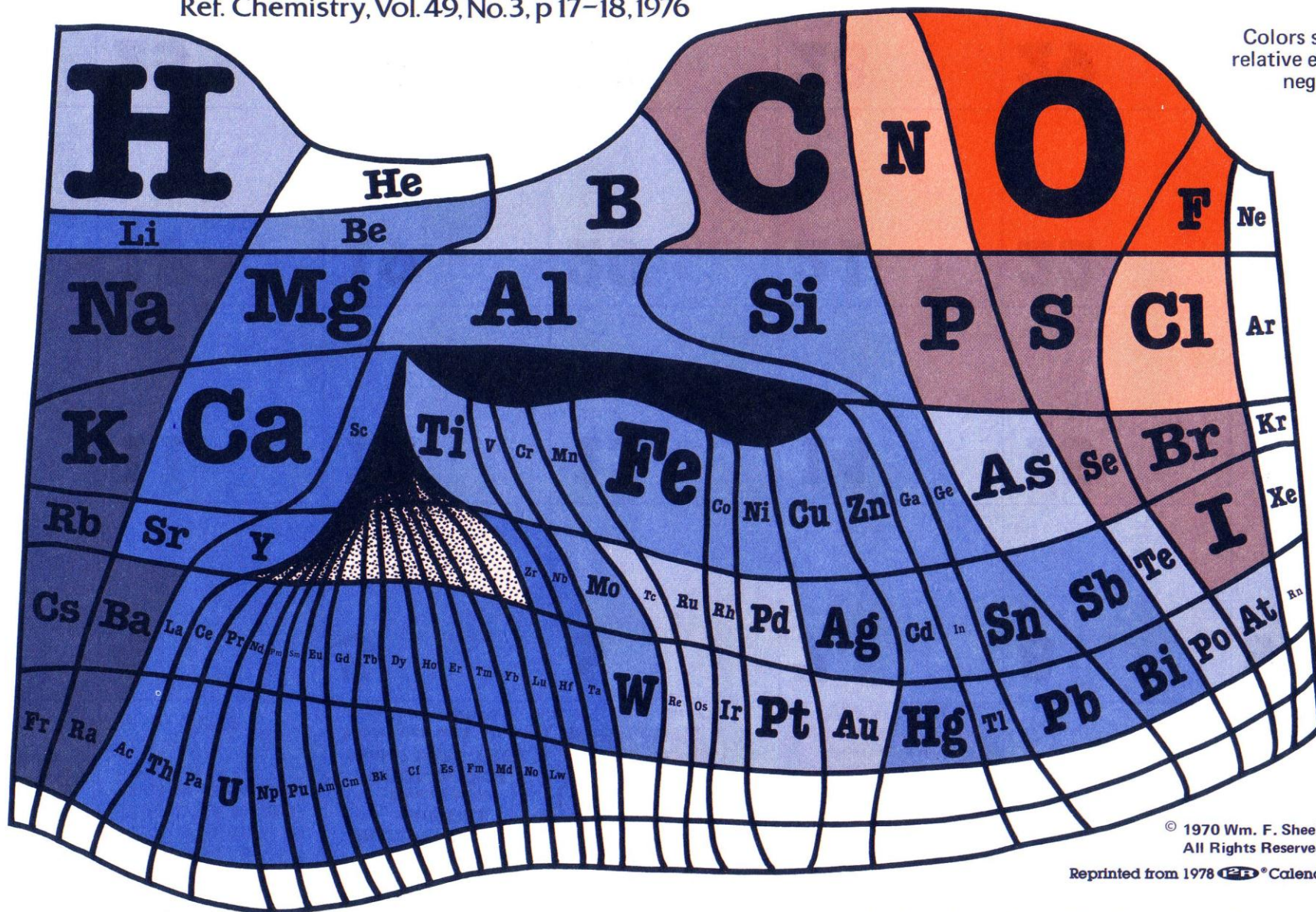


# The Elements According to Relative Abundance

A Periodic Chart by Prof. Wm. F. Sheehan, University of Santa Clara, CA 95053

Ref. Chemistry, Vol. 49, No. 3, p 17-18, 1976

Colors suggest  
relative electro-  
negativity

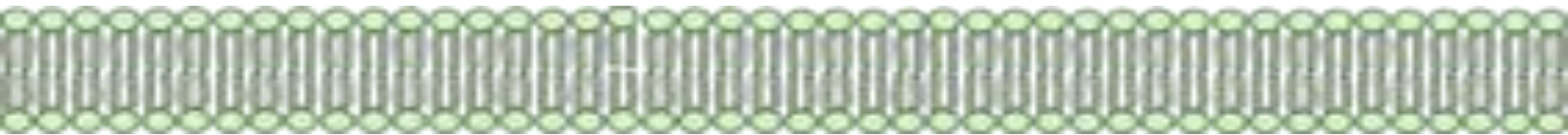
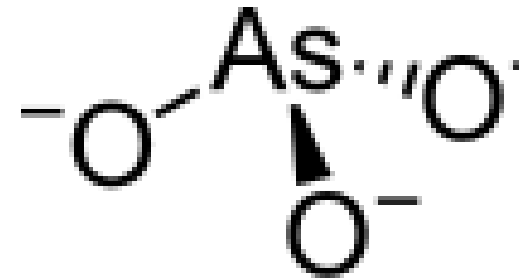
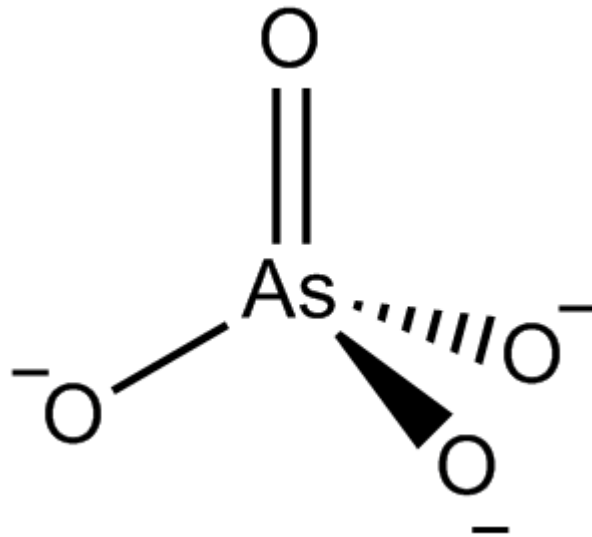
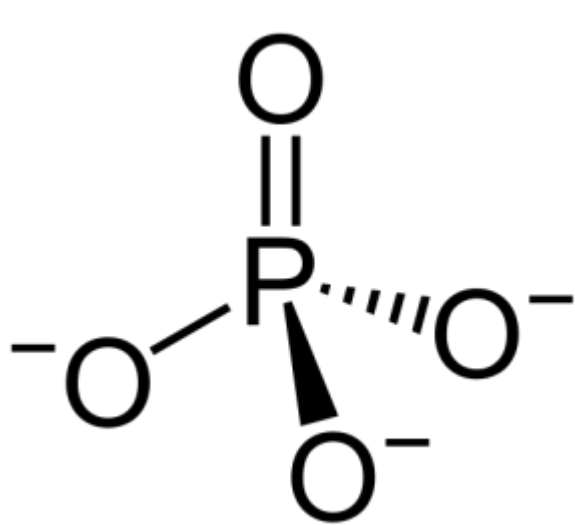


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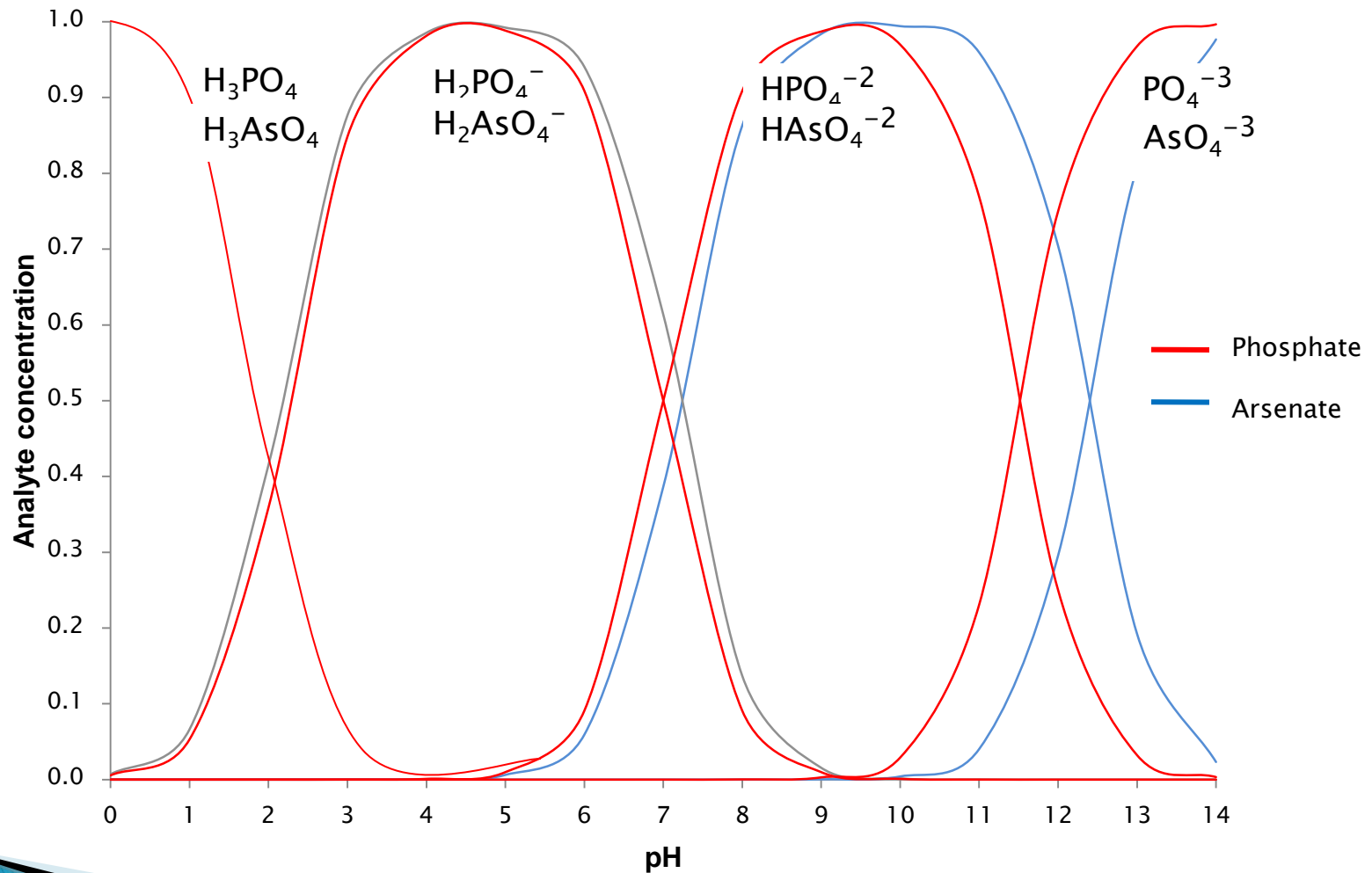
Reprinted from 1978  Calendar.



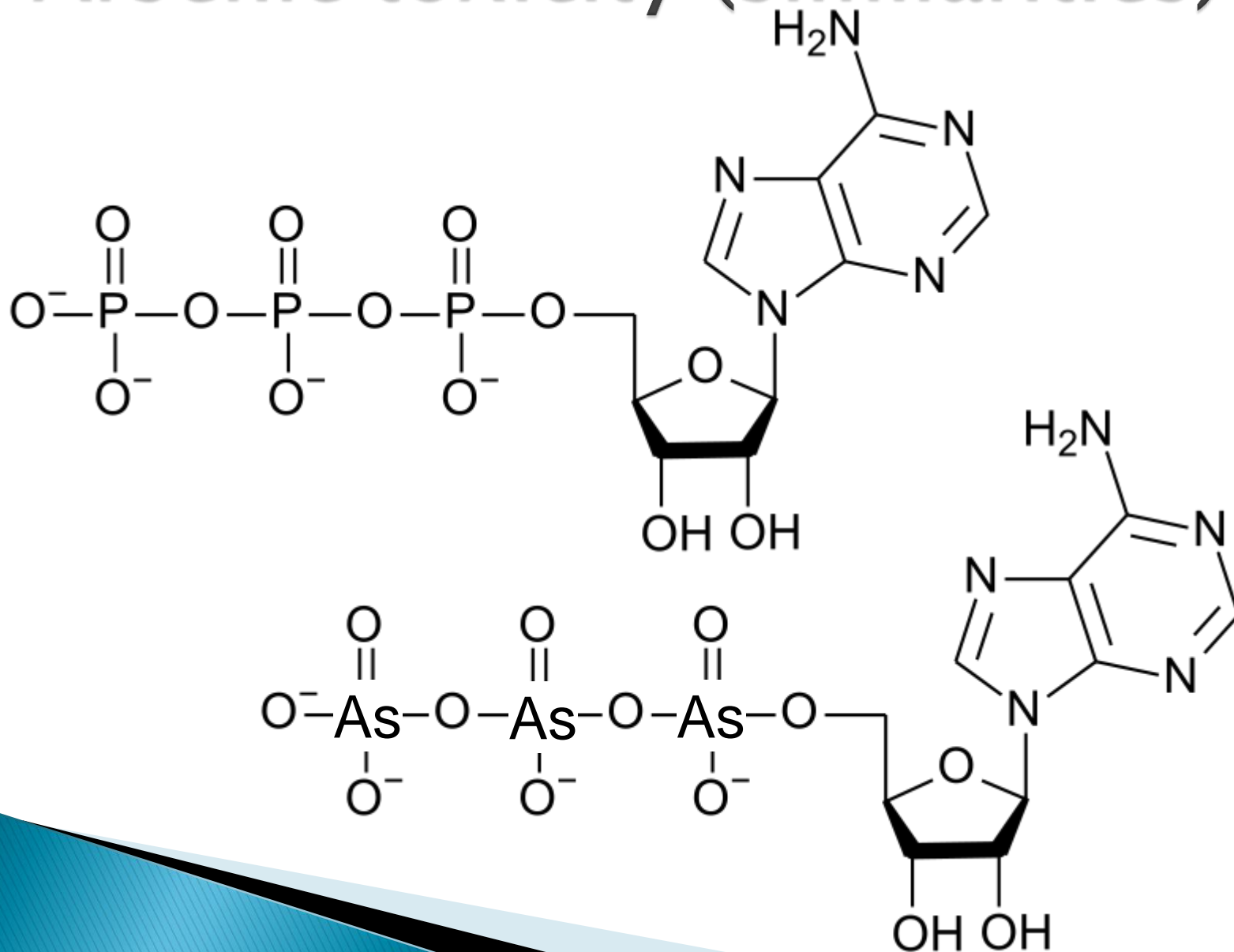
# Arsenic toxicity (similarities)



# Arsenic toxicity (similarities)



# Arsenic toxicity (similarities)



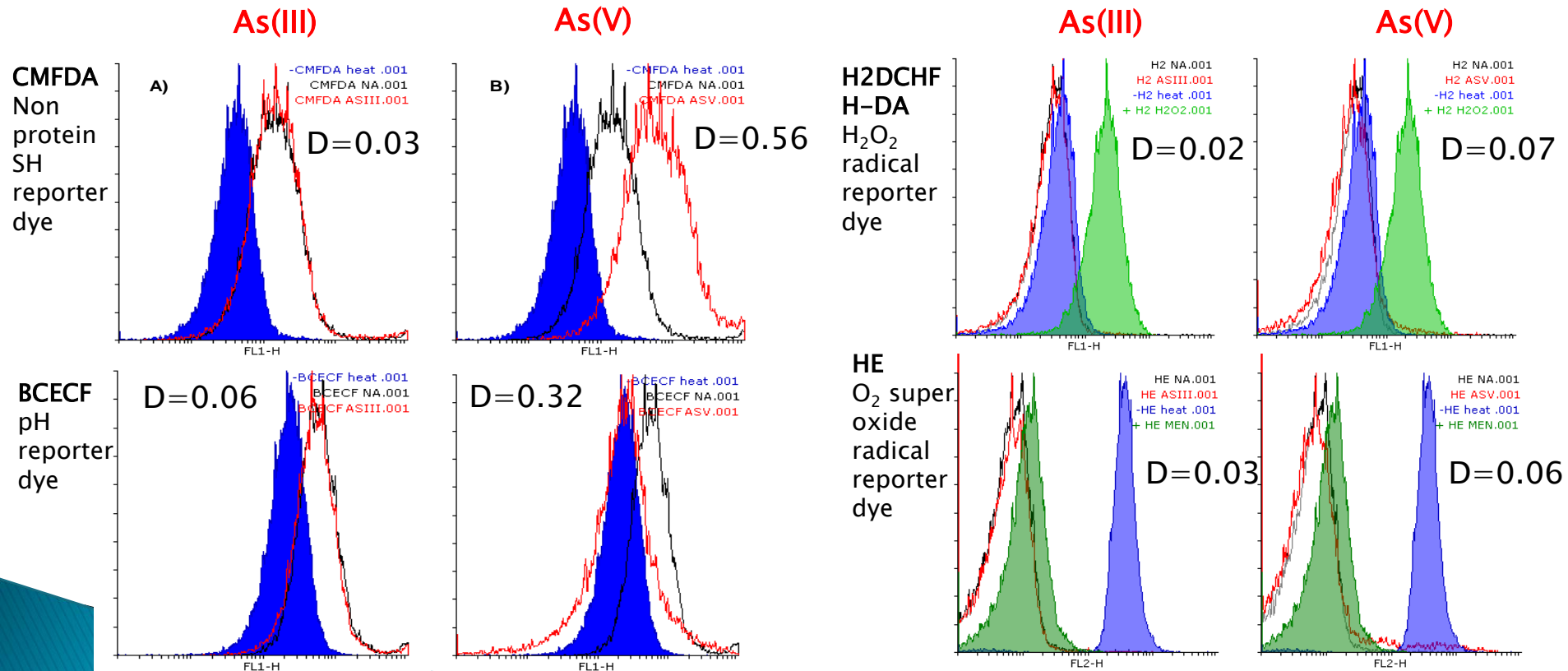
# TOXICITY OF ARSENIC TO *C. VULGARIS*



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- ▶ Toxicity of As(III) (72 h @ 0.3mg L<sup>-1</sup> phosphate) **IC<sub>50</sub>=64.23 mg L<sup>-1</sup>**
- ▶ Toxicity of As(V) (72 h @ 0.3mg L<sup>-1</sup> phosphate) **IC<sub>50</sub>=1.07 mg L<sup>-1</sup>**

Chlorophyll as  
surrogate for cell  
health



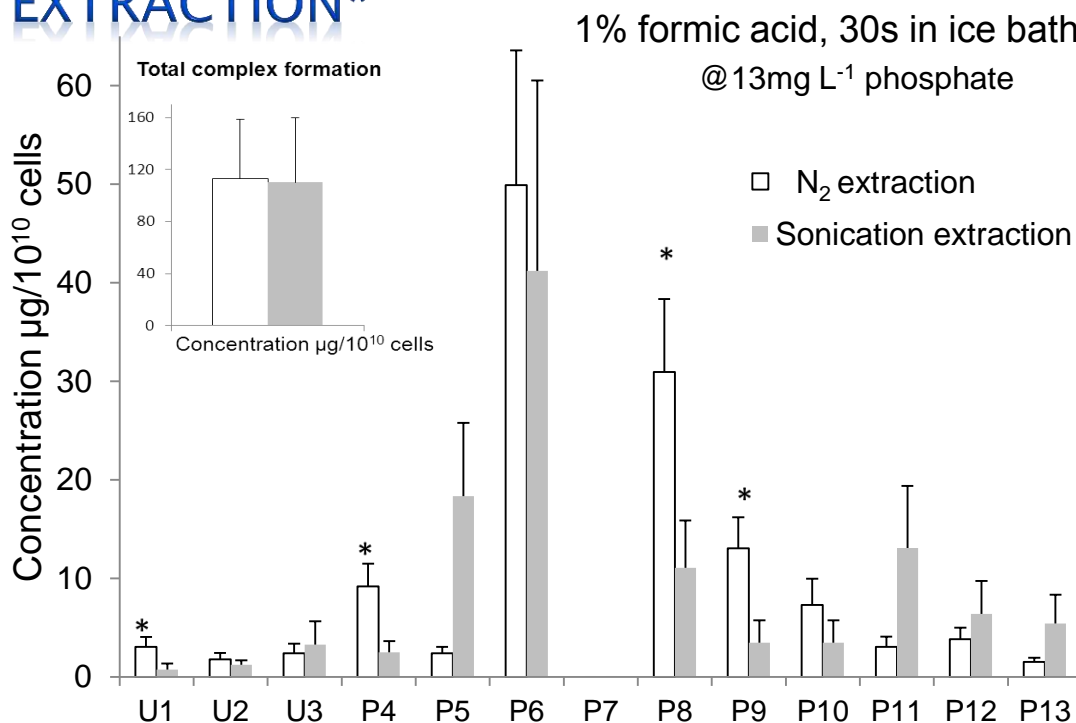
n=20,000 cells, Strong D>0.3, Moderate 0.2< D <= 0.3, Weak 0.15< D <=0.20, Negligible D <= 0.15  
Kolmogorov-Smirnov statistics



# ENHANCED DETERMINATION OF ARSENIC-PHYTOCHELATIN COMPLEXES IN *C. VULGARIS* USING FOCUSED SONICATION EXTRACTION\*



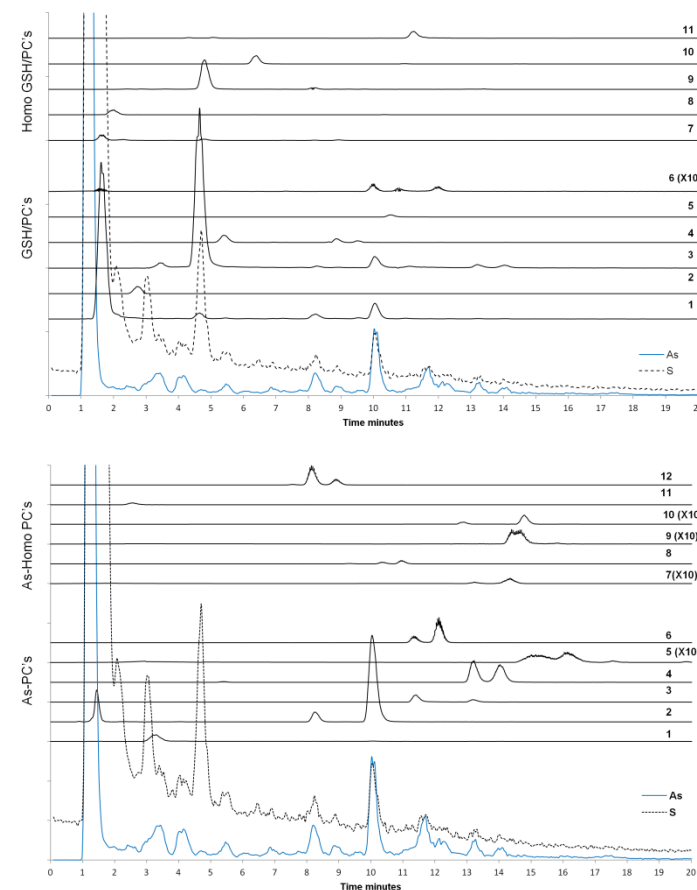
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Sample	$\mu\text{g As g}^{-1}$		% Recovery
Total extraction <i>C. vulgaris</i>	83.2	SE = 2.04, n = 6	
Sonication <i>C. vulgaris</i>	59.2	SE = 1.14, n = 6	71.10%
$\mu\text{g As L}^{-1}$			
Total extraction Kelp	23.5	SE = 0.60, n = 9	
Sonication Kelp	22.2	SE = 0.28, n = 11	94.70%
SRM 2669	48.1	%RSD = 2.16	
Certified value	50.7	$\pm 6.3$ (95% CI)	

SE= Standard error , n = Number of samples, % RSD = Relative standard deviation

## Online HPLC-ICPMS/ESMS



21 peptides

\*Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick.  
Anal. Methods, 2014,6, 791-797

# EXPOSURE TO As(III)\*

Cells exposed to 50mg L<sup>-1</sup> for 48h



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## Unbound peptides

Molecule	Formula	Monoisotopic mass (M+H <sup>+</sup> or M+2H <sup>+</sup> )	Experimental mass	Difference ppm
<b>GSH/PC</b>				
GSH	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	308.0916	308.0912	-1.34
GSSG	C <sub>20</sub> H <sub>32</sub> N <sub>6</sub> O <sub>12</sub> S <sub>2</sub>	613.1598	613.1598	0
Reduced PC <sub>2</sub>	C <sub>18</sub> H <sub>29</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub>	540.1434	540.1433	-0.16
Oxidised PC <sub>2</sub>	C <sub>18</sub> H <sub>27</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub>	538.1278	538.1289	2.04
Reduced PC <sub>3</sub>	C <sub>26</sub> H <sub>41</sub> N <sub>7</sub> O <sub>14</sub> S <sub>3</sub>	772.1952	772.1952	-0.01
Oxidised PC <sub>3</sub>	C <sub>26</sub> H <sub>39</sub> N <sub>7</sub> O <sub>14</sub> S <sub>3</sub>	770.1795	770.1795	0
Reduced PC <sub>4</sub>	C <sub>34</sub> H <sub>53</sub> N <sub>9</sub> O <sub>18</sub> S <sub>4</sub>	1004.247	Not found	
<b>Ala GSH/PC</b>				
γ-(Glu-Cys)-Ala	C <sub>11</sub> H <sub>19</sub> N <sub>3</sub> O <sub>6</sub> S	322.1073	322.1072	-0.21
γ-(Glu-Cys) <sub>2</sub> -Ala	C <sub>19</sub> H <sub>31</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub>	554.1591	554.1578	-2.2
<b>desGly GSH/PC</b>				
γ-(Glu-Cys)	C <sub>8</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub> S	251.0702	251.0706	1.55
γ-(Glu-Cys) <sub>2</sub>	C <sub>16</sub> H <sub>26</sub> N <sub>4</sub> O <sub>9</sub> S <sub>2</sub>	483.1219	483.1217	-0.58
γ-(Glu-Cys) <sub>3</sub>	C <sub>24</sub> H <sub>38</sub> N <sub>6</sub> O <sub>13</sub> S <sub>3</sub>	715.1737	715.1747	1.41

Glutathione/PC homologues (terminal amino acid Gly substituted by Ala, Ser, Gln, Glu or is absent)

## Arsenic bound peptides

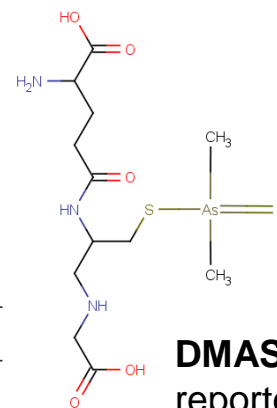
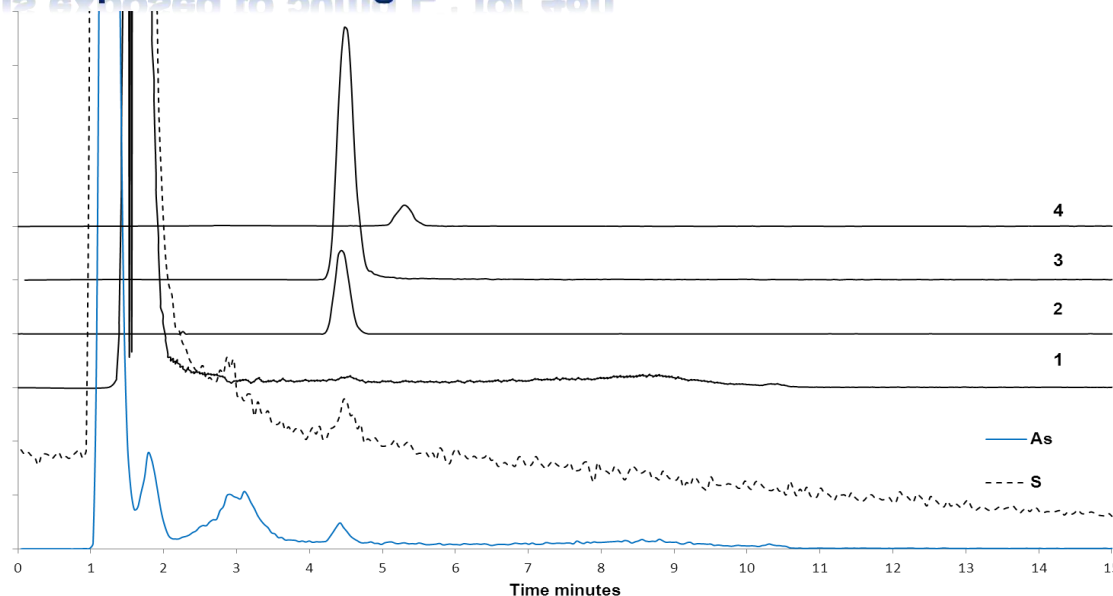
Molecule	Formula	Monoisotopic mass (M+H <sup>+</sup> or M+2H <sup>+</sup> )	Experimental mass	Difference ppm
<b>GSH/PC</b>				
As(III)-PC <sub>2</sub>	C <sub>18</sub> H <sub>28</sub> N <sub>5</sub> O <sub>11</sub> S <sub>2</sub> As	630.0443	630.0437	-0.88
As(III)-PC <sub>3</sub>	C <sub>26</sub> H <sub>38</sub> N <sub>7</sub> O <sub>14</sub> S <sub>3</sub> As	844.0933	844.0931	-0.19
GS-As(III)-PC <sub>2</sub>	C <sub>28</sub> H <sub>43</sub> N <sub>8</sub> O <sub>16</sub> S <sub>3</sub> As	460.0666	460.0663	-0.06
		919.1253	919.1247	-0.69
As(III)-(PC <sub>2</sub> ) <sub>2</sub>	C <sub>36</sub> H <sub>54</sub> N <sub>10</sub> O <sub>20</sub> S <sub>4</sub> As	576.0925	576.0923	-0.37
As(III)-PC <sub>4</sub>	C <sub>34</sub> H <sub>50</sub> N <sub>9</sub> O <sub>18</sub> S <sub>4</sub> As	1076.1451	1076.1455	0.37
MMA(III)-PC <sub>2</sub>	C <sub>19</sub> H <sub>30</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub> As	628.0728	628.0729	0.12
<b>Ala GSH/PC</b>				
As(III)-γ-(Glu-Cys) <sub>3</sub> -Ala	C <sub>27</sub> H <sub>40</sub> N <sub>7</sub> O <sub>14</sub> S <sub>3</sub> As	858.109	858.1082	-0.87
GS-As(III)-γ-(Glu-Cys) <sub>2</sub> -Ala	C <sub>29</sub> H <sub>45</sub> N <sub>8</sub> O <sub>16</sub> S <sub>3</sub> As	467.0744	467.0744	0.09
As(III)-γ-((Glu-Cys) <sub>2</sub> ) <sub>2</sub> -Ala	C <sub>37</sub> H <sub>57</sub> N <sub>10</sub> O <sub>20</sub> S <sub>4</sub> As	583.1003	583.101	1.19
MMA(III)-γ-(Glu-Cys) <sub>2</sub> -Ala	C <sub>20</sub> H <sub>32</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub> As	642.0885	642.0889	0.66
<b>desGly GSH/PC</b>				
As(III)-γ-(Glu-Cys) <sub>2</sub>	C <sub>16</sub> H <sub>25</sub> N <sub>4</sub> O <sub>10</sub> S <sub>2</sub> As	573.0306	573.0318	2.11
GS-As(III)-γ-(Glu-Cys) <sub>2</sub>	C <sub>26</sub> H <sub>40</sub> N <sub>7</sub> O <sub>15</sub> S <sub>3</sub> As	431.5558	431.5558	-0.17

Newly reported peptides

\*Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick.  
Anal. Methods, 2014,6, 791-797

# EXPOSURE TO DMA\*

Cells exposed to 50mg L<sup>-1</sup> for 48h



**DMAS<sup>V</sup>-GS** has only been reported once in *Brassica Oleracea* plants:

Cabbage, broccoli, cauliflower, kale, Brussels sprouts, collard greens, savoy, among other

1 GSH (308), 2 **DMAS<sup>V</sup>-GS (444)**, 3 Reduced PC<sub>2</sub> (540) and 4 Oxidised PC<sub>2</sub> (538), As and S signals from HR-ICP-MS

Molecule	Formula (M)	Monoisotopic mass (M+H <sup>+</sup> )	Experimental mass	Difference ppm
GSH	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	308.0916	308.0918	0.65
Red PC <sub>2</sub>	C <sub>18</sub> H <sub>29</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub>	540.1434	540.1433	-0.16
Ox PC <sub>2</sub>	C <sub>18</sub> H <sub>28</sub> N <sub>5</sub> O <sub>10</sub> S <sub>2</sub>	538.1278	538.1288	1.86
DMAS <sup>V</sup> -GS	C <sub>12</sub> H <sub>23</sub> N <sub>3</sub> O <sub>6</sub> S <sub>2</sub> As	444.0244	444.0247	0.56

@13mg L<sup>-1</sup>  
phosphate

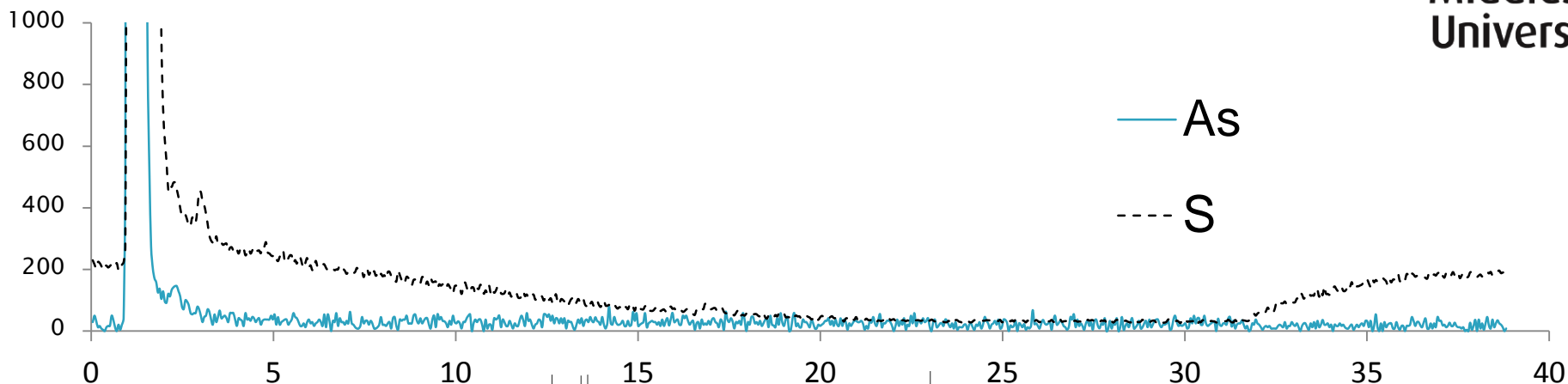
\*Leonardo Pantoja, Diane Purchase, Huw Jones, Jörg Feldmann and Hemda Garelick.  
Anal. Methods, 2014,6, 791-797

# EXPOSURE TO As(V)\*

Cells exposed to 50mg L<sup>-1</sup> for 48h

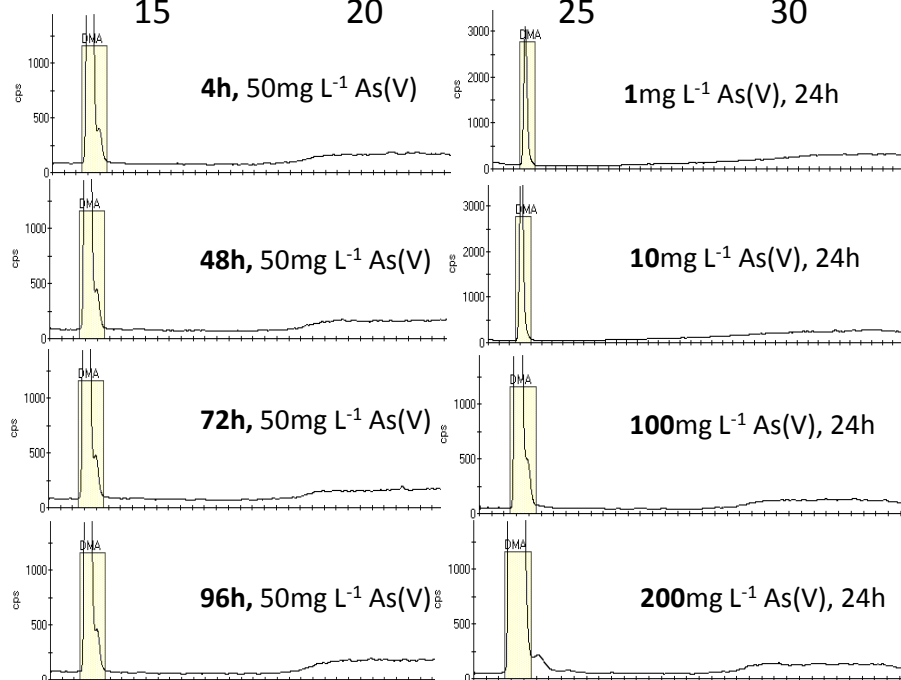


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@13mg L<sup>-1</sup> phosphate

At this level of phosphate  
cells are not under stress



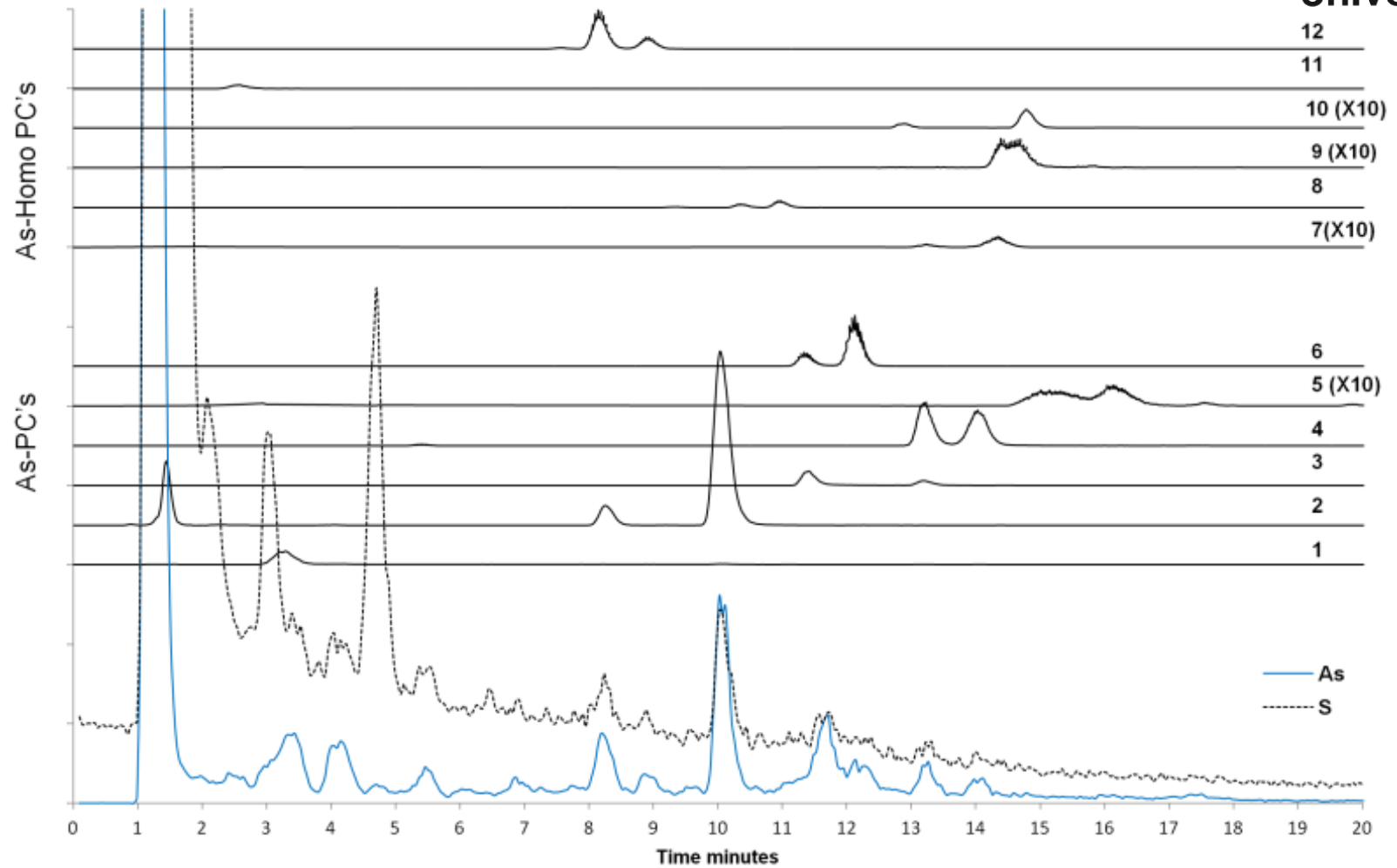
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Anal. Methods, 2014,6, 791-797



# EXPOSURE TO As(III)\*



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@13mg L<sup>-1</sup>  
phosphate

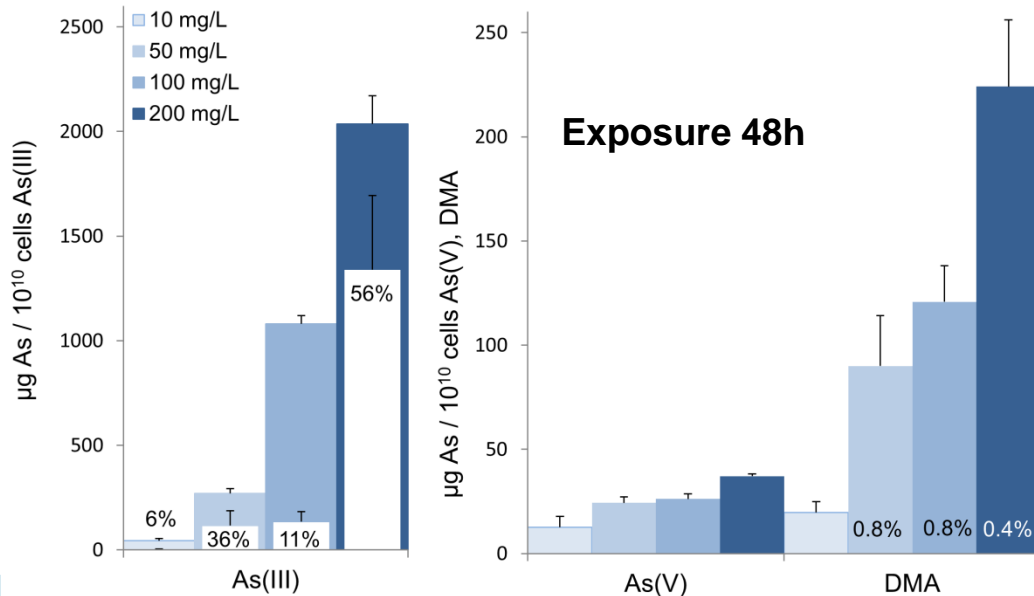
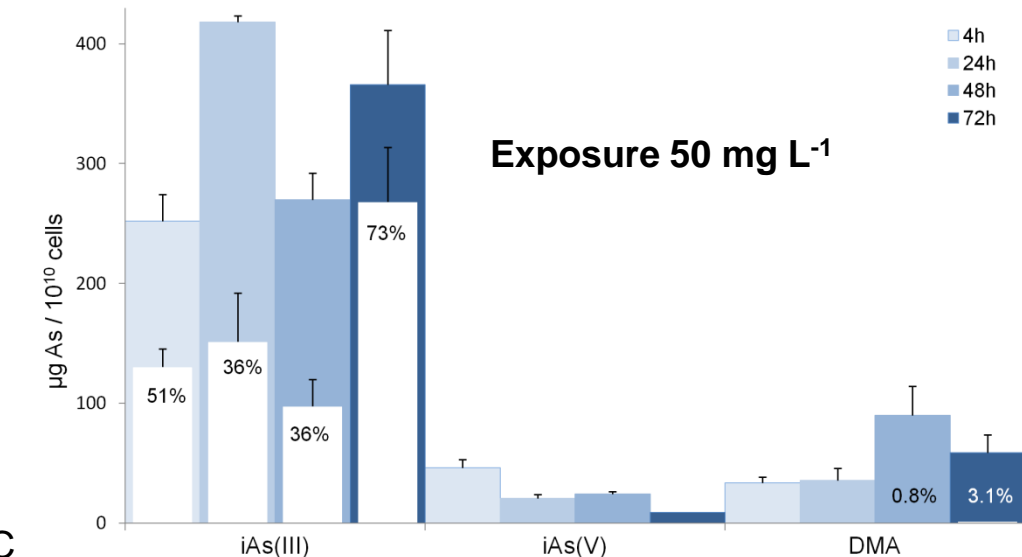
# TOTAL As-GS/PC FORMATION



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@13mg L<sup>-1</sup> phosphate

- Coloured bars - total amount of arsenic
- White bars- amount of As-GS/PC (percentage of total arsenic)



Vertical bars denote + 1 standard error, n = 3

# TRANSPORT OF As-GS/PC TO VACUOLES



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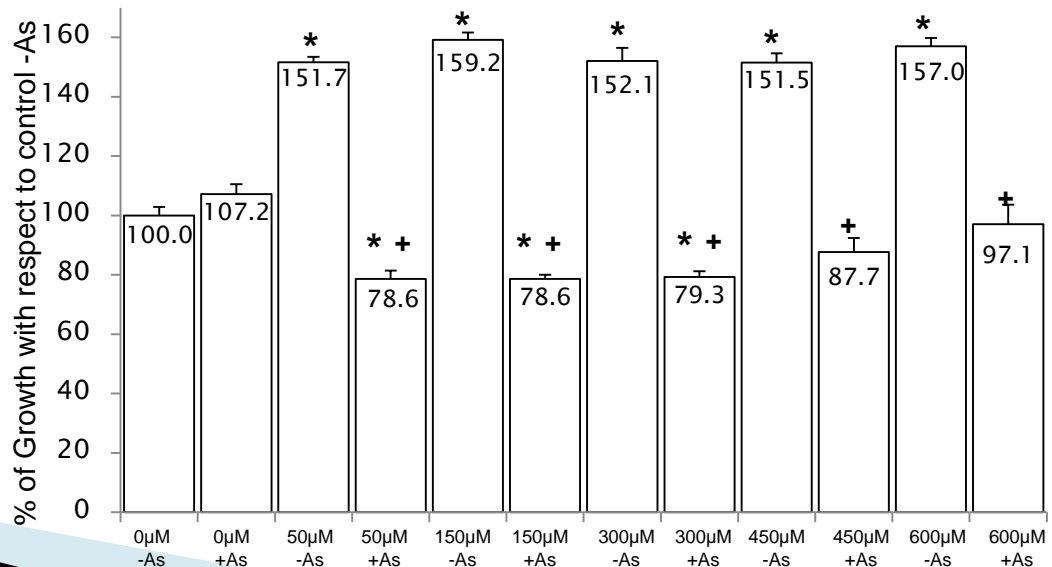
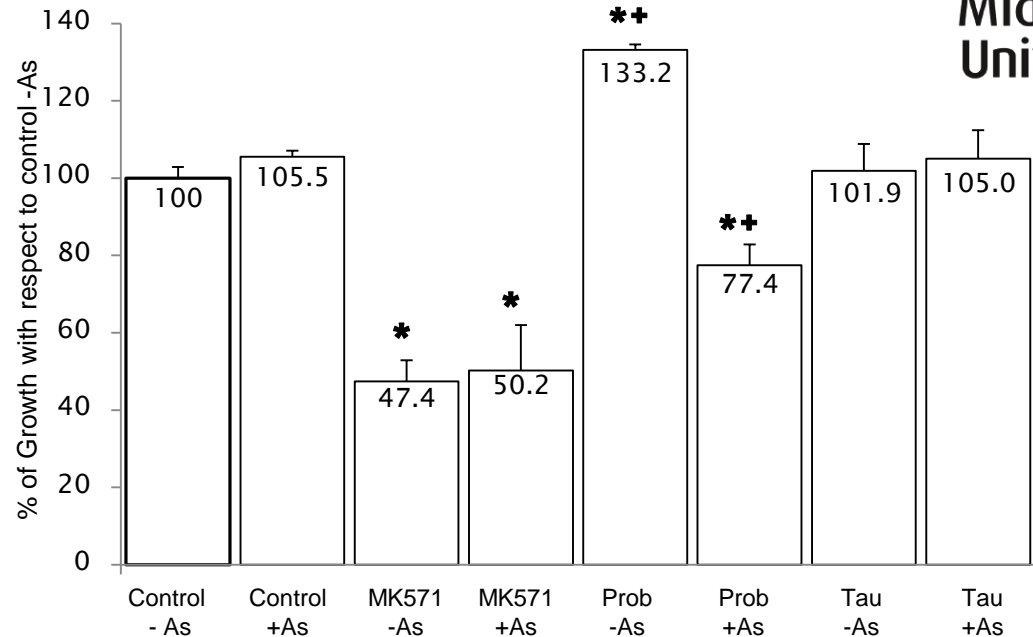
**Toxicity (72 h) to As(III) (50 mg L<sup>-1</sup>)**  
@13mg L<sup>-1</sup> phosphate

Test the presence of ABCC1 and ABCC2 inhibitors:

- MK571 (25 µM)
- Probenecid (Prob, 500 µM)
- Sodium taurocholate (Tau, 50 µM).

ABC = ATP-binding cassette transporter  
MRP = Multidrug resistance-associated protein

\* (P < 0.05) with respect to control  
+ (P < 0.05) with respect to absence/presence of arsenic  
Vertical bars + 1 standard error  
Control n=6, experiments n = 3

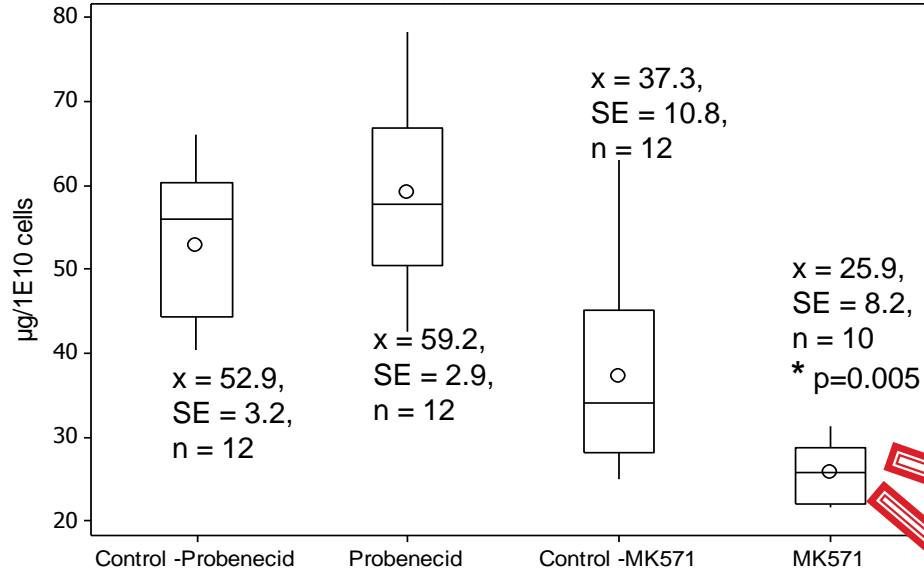


# TRANSPORT OF As-GS/PC TO VACUOLES



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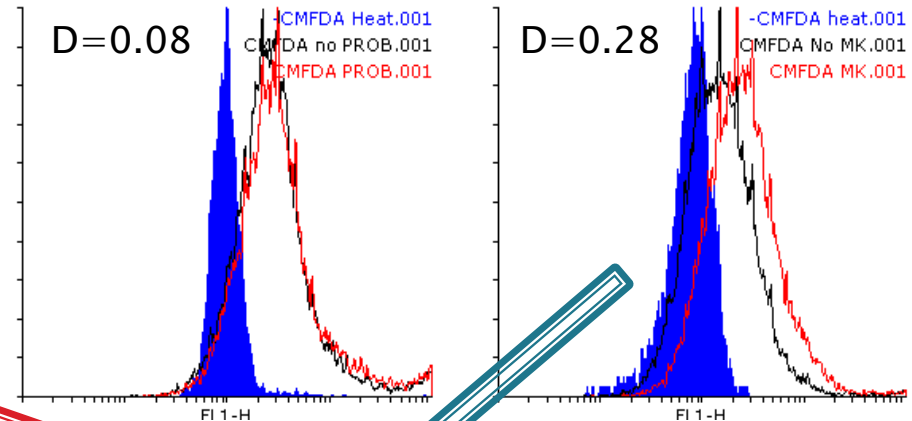
Cells exposed to 150 mg L<sup>-1</sup> of As(III) for 24 h



CMFDA substrate for ABCC transport

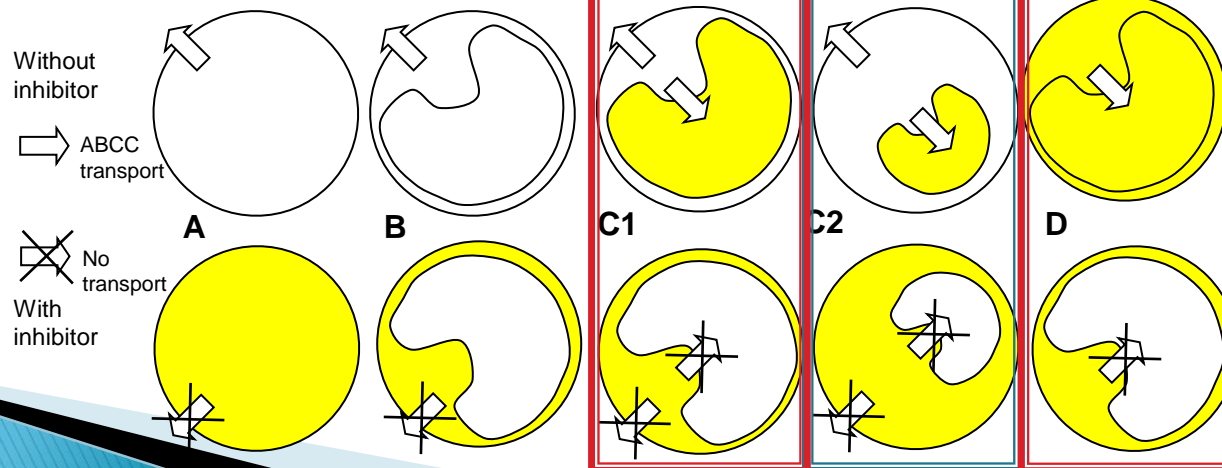
probenecid

MK571



Negligible D > 0.3, Moderate 0.2 < D ≤ 0.3, Weak 0.15 < D ≤ 0.20, Negligible D ≤ 0.15

Treatment time 60 min, n=20,000 cells





# CONCLUSIONS



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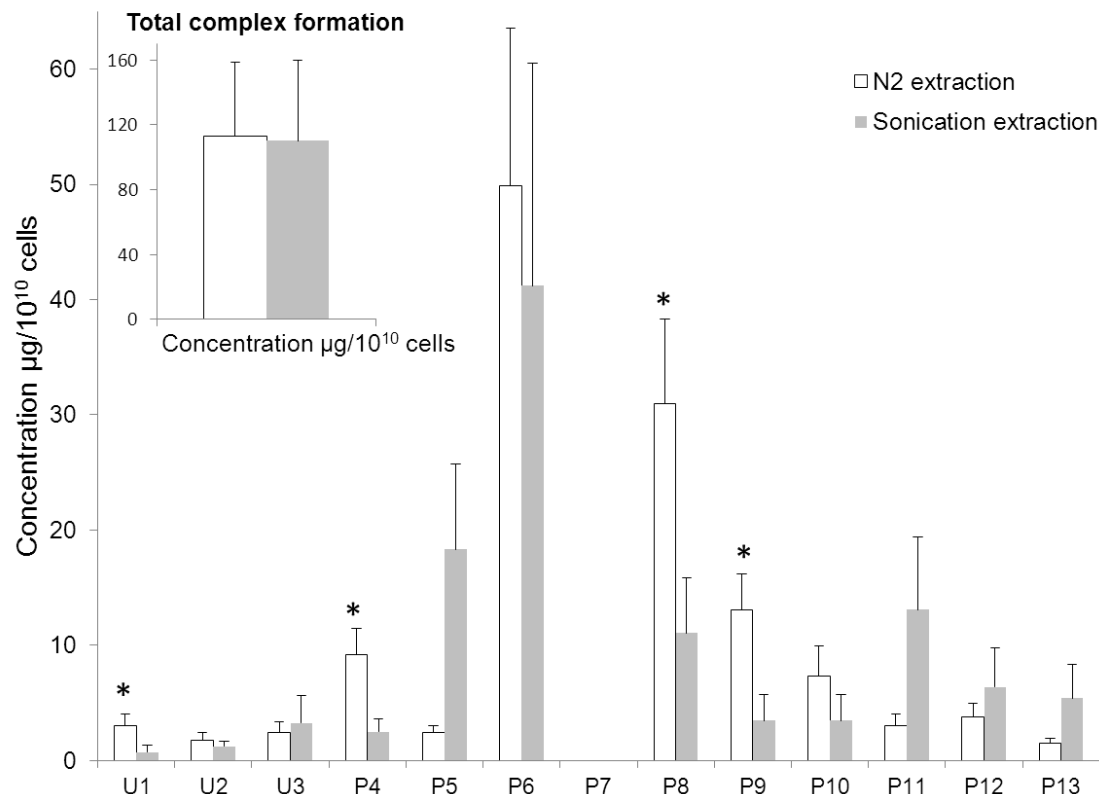
- As(V) is more toxic than As(III) to *C. vulgaris* cells at the same concentration of phosphate
- As(III) triggers the formation of **As-GS/PC** molecules
- As(V) does not trigger the formation of **As-GS/PC** molecules when cells are not under stress
- DMA triggers the formation of **DMAS<sup>V</sup>-GS** but it is unlikely that this is part of a detoxification mechanism
- ABCC1 and ABCC2 are involved in **As-GS/PC** transport to acidic vacuoles in *C. vulgaris*



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Thank you for listening.

Any questions?



**U1-3**

Unknowns

**P4**

GS-As(III)-PC<sub>2</sub>/GS-As(III)-  $\gamma$ -(Glu-Cys)<sub>2</sub>,

**P5**

As(III)-  $\gamma$ -(Glu-Cys)<sub>2</sub>

**P6**

GS-As(III)-PC<sub>2</sub>

**P7**

GS-As(III)-  $\gamma$ -(Glu-Cys)<sub>2</sub>-Ala

**P8**

As(III)-PC<sub>3</sub>/ MMA(III)-PC<sub>2</sub>

**P9**

MMA(III)-PC<sub>2</sub>

**P10**

As(III)-PC<sub>3</sub>/ As(III)-(PC<sub>2</sub>)<sub>2</sub>

**P11**

As(III)-(PC<sub>2</sub>)<sub>2</sub>/ As(III)- $\gamma$ -(Glu-Cys)<sub>3</sub>-Ala/ As(III)-  $\gamma$ -((Glu-Cys)<sub>2</sub>)<sub>2</sub>-Ala/

**P12**

MMA(III)-  $\gamma$ -(Glu-Cys)<sub>2</sub>-Ala,

**P13**

As(III)-PC<sub>4</sub>